

**Title:** Evaluation of Enzyme Linked Immunosorbent Assay (ELISA) for Diaminoatrazine Analysis of Water Samples in Comparison to Gas Chromatography

**Project I.D.:** DNR Project #175

**Investigators:**

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**Period of Contract:** 7/1/02-6/30/03

**Background/Need:** Long term use of the herbicide atrazine in Wisconsin has resulted in the contamination of Wisconsin's groundwater. While atrazine has been restricted or banned in certain areas susceptible to groundwater contamination, it is still being used in other areas as an effective means to control weeds in corn crops. While the detection of atrazine led to the original environmental concerns, it has also been recognized that degradation products or metabolites of atrazine can exist in groundwater. One of the metabolites, diaminoatrazine, is of particular concern because of its toxicity. However, analytical techniques to measure this metabolite have been time consuming and expensive.

**Objectives:** A new test for diaminoatrazine, employing an enzyme linked immunosorbent assay (ELISA), is evaluated as a groundwater-monitoring tool.

**Methods:** Seventy four water samples, mostly groundwater, were analyzed for atrazine and atrazine metabolites using conventional gas chromatographic methods. Aliquots of the 74 samples were also analyzed using both a diaminoatrazine and atrazine (an ELISA for atrazine has been in use for many years). An evaluation of the ELISA results, relative to the conventional chromatographic measurements, was then made. The majority of the water samples were collected from wells with a history of atrazine and atrazine metabolite contamination.

**Results and Discussion:** While the new diaminoatrazine ELISA correlates with conventional gas chromatographic measurements of diaminoatrazine, the ELISA produced results that were generally higher (about double) than those obtained from gas chromatography. The higher ELISA results are probably due to the fact that other triazine compounds, including the parent atrazine, can positively bias the diaminoatrazine ELISA (i.e., the specificity for diaminoatrazine is not 100 percent). At the same time the ELISA for diaminoatrazine can detect lower levels of diaminoatrazine than conventional gas chromatography. Some samples in which neither atrazine nor any of its metabolites could be detected by conventional gas chromatography had detects using the diaminoatrazine ELISA. Total atrazine measured by the conventional GC technique (sum of the parent compound concentration and the concentration of all detected metabolites) gave similar results to the sum of the diaminoatrazine ELISA concentration and the older atrazine ELISA for the samples studied.

**Conclusions/Implications/Recommendations:** Overall, the new diaminoatrazine ELISA is straightforward to perform as the test follows similar procedures as other ELISAs. The test is inexpensive to perform compared to conventional gas chromatographic procedures. Interpretation of the results is complicated by the fact that other triazine compounds, including the parent compound atrazine, can cross react. Nevertheless, given the public health concern for diaminoatrazine, the ELISA test, which was found to respond to very low concentrations of diaminoatrazine, might be used as a valuable screening tool or as a supplemental analysis.

**Related Publications:** None.

**Key Words:** triazine, atrazine, diaminoatrazine, ELISA, groundwater,

**Funding:** Wisconsin DNR; State of Wisconsin Groundwater Research & Monitoring Program

**Final Report:** A final report containing more detailed information on this project is available for loan at the Water Resources Institute Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.